IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re:	Application of:	Hiroyuki ABURATANI et al. Confirmation No. 4237
	Serial No.:	10/526,508
	Filed:	September 4, 2003
	For:	**A METHOD FOR DIAGNOSING CANCER BY DETECTING GPC3**
	Examiner:	Catherine JOYCE
	Art Unit:	1642

DECLARATION UNDER 37 CFR 1.132

I, Dr. Tadashi MATSUURA , the undersigned, being a citizen of Japan, hereby declare the following:

WHEREAS, I currently hold the position of Manager of Business Development at Perseus Proteomics Inc., located at 4-7-6 Komaba, Meguro-ku, Tokyo 153-0041 JAPAN, and have held this position since 2005.

WHEREAS, Perseus Proteomics is the assignee of the above-identified patent application pending before the United States Patent and Trademark Office.

WHEREAS, prior to my current position, I held the following professional positions:

2003-2005	General Manager of Research Development, HuBit Genomix Inc.
2002-2003	Researcher, HuBit Genomix Inc.
1997- 2002	Researcher, Institute of Molecular Cellular Biology, National Institute of Advanced Industrial Science and Technology (AIST) (former name was "National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, MITI")
1992-1997	Research Associate, Departments of Medicine (Neurology) and Microbiology, Dartmouth Medical School, Hanover, NH
1988-1994	Research Associate, Department of Parasitology, Shinshu University School of Medicine, Japan.

WHEREAS, my educational background includes:

1986-1988 Postdoctoral Researcher in Department of Parasitology, Shinshu University School of Medicine, Japan

Apr-Sep 1986 Postdoctoral Researcher in Developmental Biology, Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan (Mitsubioshi-Kasei Industry Inc.) under director Dr. Higashinakagawa.

1983-1986 Graduate student (Doctorate course) at Tokyo Metropolitan University, Tokyo, Japan. Department of Developmental Biology under Associate Professor Toru Higashinakagawa
Thesis: "Studies on the control of transcription of *Tetrahymena* ribosomal RNA gene" Awarded D. Sc. (Tokyo Metropolitan University) in Biology, conferred March 1986.

1981-1983 Graduate student (Master course) at Kobe University, Department of Microbiology and Biochemistry under Professor Takeharu Kanehisa. Actual Research performed at Department of Molecular Biology, University of Occupational and Environmental Health School of Medicine, Kitakyushu, Japan in collaboration with Professor Takashi Mita & Associate Professor Toru Higashinakagawa. Research Project; Structure and expression of *Tetrahymena* ribosomal DNA.
M.Sc. in Biology, March, 1983.
M.Sc. dissertation on "Development of the *in vitro* transcription system with *Tetrahymena* rDNA clone".

1980-1981 Undergraduate Study, Laboratory of Developmental Biology , Department of Biology, Faculty of Science, Shinshu University.
B.Sc. in Biology, March, 1981.
B.Sc. dissertation on "Electrophoretic analysis of egg proteins in *Carassius auratus langsdorfii*" under the supervision of Professor Takao Kajishima.

WHEREAS, I have authored or co-authored research papers published in scientific journals, including peer-reviewed scientific journals, relating to gene expression or variation in hepatic or other disease, which include the following:

Kato N, Ji G, Wang Y, Baba M, Hoshida Y, Otsuka M, Taniguchi H, Moriyama M, Dharel N, Goto T, Shao RX, Matsuura T, Ishii K, Shiina S, Kawabe T, Muramatsu M, Omata M. Large-scale search of single nucleotide polymorphisms for hepatocellular carcinoma susceptibility genes in patients with hepatitis C. Hepatology. 2005 Oct;42(4):846-53.

Takabatake N, Sata M, Inoue S, Shibata Y, Abe S, Wada T, Machiya J, Ji G, Matsuura T, Takeishi Y, Muramatsu M, Kubota I. A Novel Polymorphism in Secretory Phospholipase A2-IID Is Associated with Body Weight Loss in Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2005 Nov 1;172(9):1097-104. Epub 2005 Jul 7.

Saito T, Ji G, Shinzawa H, Okumoto K, Hattori E, Adachi T, Takeda T, Sugahara K, Ito JI, Watanabe H, Saito K, Togashi H, Ishii K, Matsuura T, Inageda K, Muramatsu M, Kawata S. Genetic variations in humans associated with differences in the course of hepatitis C. Biochem Biophys Res Commun. (2004) 317, 335-41.

Daimon M, Ji G, Saitoh T, Oizumi T, Tominaga M, Nakamura T, Ishii K, Matsuura T, Inageda Matsumine H, Kido T, Htay L, Kamatani N, Muramatsu M, Kato T. Large-scale search of SNPs for type 2 DM susceptibility genes in a Japanese population. Biochem Biophys Res Commun. (2003) 302, 751-8.

Sutou, S. Miwa, K, Matsuura, T, Kawasaki, Y, Ohinata, Y and Mitsui Y. Native Tesmin Is a 60-Kilodalton Protein That Undergoes Dynamic Changes in Its Localization During Spermatogenesis in Mice. Biol Reprod (2003);68, 1861-9.

Takenaka Y, Haga N, Harumoto, Matsuura T, Mitsui Y. Transformation of Paramecium caudatum with a novel expression vector harboring codon-optimized GFP gene. Gene (2002) 284, 233-40.

Takenaka, Y, Matsuura T, Haga N, Mitsui T. Expression of telomerase reverse transcriptase and telomere elongation during sexual maturation in Paramecium caudatum. Gene (2001)264, 153-61.

Matsuura, T., Kawasaki, Y., Miwa, K., Sutou, S., Ohinata, Y., Yoshida, F. & Youji, M. Germ cell-specific nucleocytoplasmic shuttling protein, Tesmin, responsive to heavy metal stress in mouse testis. J Inorg Biochem. (2002) 88, 183-91.

Lee YH, Channon JY, Matsuura T, Schwartzman JD, Shin DW, Kasper LH. Functional and quantitative analysis of splenic T-cell immune responses following oral toxoplasma gondii infection in mice. Exp Parasitol (1999) 91, 212-21

Khan IA, Matsuura T, Kasper LH. Inducible nitric oxide synthase is not required for long-term vaccine-based immunity against Toxoplasma gondii. J Immunol (1998) 161, 2994-3000

Matsuura, T, Khan. I. & Kasper, L. cDNA structure of p97 antigen against which monoclonal antibody inhibites multiplication of*Toxoplasma gondii* in host cell. Mol Biochem Parasitol (1997) 90, 403-13

Khan IA, Schwartzman JD, Matsuura T, Kasper LH. A dichotomous role for nitric oxide during acute *Toxoplasma gondii* infection in mice. Proc Natl Acad Sci U S A (1997) 94, 13955-60.

Kasper, L.H., Matsuura, T., Fonseka, S., Arruda, J. & Khan, I.A. (1996) Induction of T cells during acute murine infection with Toxoplasma gondii. J. Immunol. 157, 5521-5527

Khan, I.A., Matsuura, T., Fonseka, S. & Kasper, L. (1996) Production of Nitric Oxide (NO) is not essential for protection against acute *Toxoplasma gondii* infection infection in IRF-1$^{-/-}$ mice. J. Immunol. 156, 636-643.

Khan, I.A., Matsuura, T. & Kasper, L. (1996) Activation-mediated CD4^{+} T cell unresponsiveness during acute *Toxoplasma gondii* infection in mice. International Immunology, 8, 887-896.

Kasper, L., Matsuura, T. & Khan, I.A. (1995) IL-7 stimulates protective immunity in mice against the intracellular pathogen, *Toxoplasma gondii*. J. Immunol. 155, 4798-4804.

Khan, I.A., Matsuura, T. & Kasper, L. (1995) IL-10 mediates immunosuppression following primary infection with *Toxoplasma gondii* in mice. Parasite Immunology, 17, 185-195.

Khan, I.A., Matsuura, T. & Kasper, L. (1994) IL-12 enhances murine survival against acute Toxoplasmosis. Infection and Immunity 60, 2908-2916.

Matsuura, T., Tegoshi, T., Furuta-Matsuura, M. & Sugane, K.(1992) Epitope-selected monospecific antibodies to recombinant antigens from *Toxoplasma gondii* reacted with dense granules of tachyzoite. J. Histochem. Cytochem. 40, 1725-1730.

Matsuura, T. & Higashinakagawa, T. (1992) *In vitro* transcription in isolated nucleoli of Tetrahymena pyriformis. Developmental Genetics. 13, 143-150.

Matsuura, T., Bylund, G. & Sugane, K. (1992) Comparison of restriction fragment length polymorphisms of ribosomal DNA between *Diphyllobothrium nihonkaiense* and *Diphyllobothrium latum*. J. Helminthol. 66, 261-266.

Matsuura, T., Sun, S. & Sugane, K. (1992) The identity of *Anisakis* typeII larvae with *Anisakis physeteris* confirmed by restriction fragment length polymorphism analysis of genomic DNA. J. Helminthol. 66, 33-37.

Sugane, K., Sun, SH. & Matsuura, T. (1992) Radiolabeling of the exceretory and somatic antigens of *Anisakis simplex* larvae. J Helminthol. 66, 305-309

Sugane, K. Sun, SH & Matsuura, T (1992) Molecular cloning of the cDNA 42 kDa antigenic polypeptide of *Anisakis simplex* larvae. J Helminthol. 66, 25-32.

Sun, S., Matsuura, T. & Sugane, K. (1992) Stage-specific expression of 34 kilodalton antigen in *Dirofilaria Immitis*. J. Helminthol.66, 62-67.

Sun, S., Matsuura, T. & Sugane, K. (1991) Molecular cloning of the cDNA encoding an immunodominant antigen of *Dirofilaria immitis*. J. Helminthol. 65, 149-158.

Sugane, K., Liu, Q., & Matsuura, T. (1989) Restriction fragment length polymorphism of Anisakinae larvae. J. Helminthol. 63, 269-274.

Sugane, K., Matsuura, T. & Maekawa, H. (1987) Translation products of mRNA from infective larvae of *Trichinella spiralis* include an antigenic polypeptide. J. Helminthol. 61, 1-8.

Matsuura, T., Matsui, T., Saiga, H., Mita, T. & Higashinakagawa, T. (1985) Faithful initiation of the *in vitro* transcription of a cloned rDNA fragment from *Tetrahymena pyriformis*. Gene 49, 225-233. (Dr. thesis)

NOW THEREFORE, having been established as an expert in the field of gene expression in hepatic disease, and having read and understood the currently outstanding Office Action in the above-identified patent application, I declare the following:

THAT, Appendix A, attached hereto and hereby incorporated by reference in this Declaration, describes the experimental protocol undertaken by me, or at my direction, wherein levels of serum GPC3 were measured in hepatic cancer patients and compared to normal controls. The data presented in Appendix A is from GPC3 protein concentration from serum of normal human control and patients with hepatic cancer

THAT, the results from this experiment, a summary of which is included in Appendix A, demonstrate that over-expression of soluble GPC3 is frequently detected in human patients with hepatic cancer. Thus, increased serum levels of GPC3, as compared to normal controls, can predictably detect hepatic cancer in humans.

I HEREBY DECLARE THAT ALL STATEMENTS MADE HEREIN OF MY OWN KNOWLEDGE ARE TRUE AND THAT ALL STATEMENT MADE ON INFORMATION AND BELIEF ARE BELIEVED TO BE TRUE; AND FURTHER THAT THESE STATEMENTS WERE MADE WITH THE KNOWLEDGE THAT WILLFUL FALSE STATEMENTS AND THE LIKE SO MADE ARE PUNISHABLE BY FINE OR IMPRISONMENT, OR BOTH, UNDER 18 U.S.C. 1001 AND THAT SUCH WILLFUL FALSE STATEMENTS MAY JEOPARDIZE THE VALIDITY OF THE PATENT APPLICATION OR ANY PATENT ISSUED THEREON.

Further, Declarant sayeth naught.

Date: Aug 4, 2008 By: 松浦 正 (TADASHI MATSUURA)

Dr. Tadashi MATSUURA
Manager of Business Development
Perseus Proteomics Inc.

APPENDIX A

INCREASED GPC3 LEVELS IN SERUM USED TO DETECT HEPATIC CANCER IN HUMANS

Serum samples

172 serum samples of Hepatic Cell Cancer (HCC) patients were collected at Tokyo University, and 80 normal controls were collected at medical examination after excluding patients with liver dysfunctions.

The population consisted of patients who had undergone curative hepatic resection for HCC at Tokyo University Hospital. The diagnosis of HCC was based on the general method; CT (computed tomography), MRI (magnetic resonance imaging), angiography, serum AFP, liver biopsy, etc.

The indication of surgical resection and operative procedure was determined according to the decision criteria based on the presence or absence of ascites, serum bilirubin level, and indocyanine green retention rate at 15 min.

ELISA

Glypican 3 (GPC3) sandwich ELISA

Monoclonal antibodies for glypican 3 were generated by the previously described method. Briefly, we used recombinant glypican 3 lacking glycosylated phosphatidylinositol (GPI)-anchoring domain, GPC3GPI as an immunogen. Ten μg /mL of anti-GPC3 polyclonal antibody per well was immobilized to 96-well plate Maxisorp (Nalge Nunc International, Roskilde, Denmark) and stabilized with Immunoassay Stabilizer (Advanced Biotechnologies Inc., Columbia, MD). Fifty μl of sera or standard were diluted with 50 μl of buffer containing 20% normal rabbit serum (Pel-Freez Biologicals, Rogers, AR), 1% BSA (Oriental Yeast Co., Ltd., Osaka, Japan), and 2% mouse ascites Hyb-3423 (Institute of Immunology, Tokyo, Japan) in 50 mM Tris-Cl (pH 8.0), 0.15 M NaCl, and 1 mM EDTA and incubated at room temperature for 2 h. 25 μl of biotinylated antibody solution containing anti-GPC3 monoclonal antibody M18D04 (20 μg/ml) and incubated at room temperature for 2 h. Discard reaction mixture, and 100 μl of horseradish peroxidase-labeled streptavidin (Vector Laboratories Inc., Burlingame, CA) were added to the plate and incubated twice at room temperature for 30 min. After washing, TMB Soluble Reagent and Stop Buffer (Scy Tek Laboratories, Inc., Logan, UT) were added as substrate, and absorbance at 450 nm was read with EIA Reader (Corona Electric Co., Ltd., Ibaraki, Japan). Recombinant GPC3GPI was used as a standard sample in each assay.

OOO was immobilized on a 96-well immunoplate was coated with the anti-GPC3 antibody diluted using a coating buffer (0.1 M NaHCO.sub.3 (pH 9.6), 0.02% (w/v) NaN.sub.3) at 10 .mu.g/mL, followed by incubation overnight at 4.degree. C. On the next day, the plate was washed 3 times with 300 .mu.L/well washing buffer (0.05% (v/v) Tween20, PBS), and then 200 .mu.L of dilution buffer (50 mM Tris-HCl (pH8.1), 1 mM MgCl.sub.2, 150 mM NaCl, 0.05% (v/v) Tween20, 0.02% (w/v) NaN.sub.3, 1% (w/v) BSA) was added for blocking. The plate was stand at room temperature for few hours or at 4.degree. C. overnight, the mouse plasma or the culture supernatant appropriately diluted with a dilution buffer was added, and incubated at room temperature for 1 hour. After washing 3 times with 300 .mu.L/well of ??, biotin-labeled anti-GPC3 antibodies diluted with a dilution buffer at 10 .mu.g/mL were added, and incubated at room temperature for 1 hour. After washing 3 times with 300 .mu.L/well of RB, AP-streptavidin (ZYMED) diluted to 1/1000 with a dilution buffer was added, and incubated at room temperature for 1 hour. After washing 5 times with 300 .mu.L/well washing buffer, color development was performed using AMPAK (DAKO CAT#K6200) according to the attached protocols. Absorbance at 450nm was then measured using a microplate reader.

Results and Conclusion

Mean ± SD of normal controls was 0.051±0.019. Cut off value was determined by adding 3 SD to mean value of normal controls. Using this cut off value of 0.108, 102 of 172 HCC cases were identified as having increased GPC3 serum levels as compared to normal controls.